

AMENDMENTS TO THE CLAIMS:

Claims 1, 2, 4, 5, 7-20, 22, 23, 59, and 61 have been canceled without prejudice or disclaimer. Claims 62-91 have been added. The following is the status of the claims of the above-captioned application, as amended.

Claims 1-61 (Canceled)

Claim 62 (New). A method for producing a polypeptide, comprising:

(a) culturing a bacterial host cell comprising two or more amplified copies of an amplification unit in the chromosome, said amplification unit comprising:

i) an expression cassette comprising at least one copy of a gene of interest encoding the polypeptide; and

ii) at least one expressible copy of a chromosomal gene of the host cell encoding at least one enzyme involved in the removal of UDP-galactose from the bacterial cell when the cell is grown in the presence of galactose or a galactose precursor; and

(b) recovering the polypeptide.

Claim 63 (New). The method of claim 62, wherein the host cell is a *Bacillus* cell selected from the group consisting of *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*.

Claim 64 (New). The method of claim 62, wherein the at least one expressible copy of the chromosomal gene of step (a) encodes an enzyme selected from the group consisting of galactokinase (EC 2.7.1.6), UTP-dependent pyrophosphorylase (EC 2.7.7.10), UDP-glucose-dependent uridylyltransferase (EC 2.7.7.12), and UDP-galactose epimerase (EC 5.1.2.3).

Claim 65 (New). The method of claim 62, wherein the at least one expressible copy of the chromosomal gene of step (a) is galE.

Claim 66 (New). The method of claim 62, wherein the galactose precursor is lactose, melibiose, raffinose, stachyos, verbascose or galactinol.

Claim 67 (New). The method of claim 62, wherein the bacterial host cell is cultured in a medium comprising an enzyme capable of degrading the galactose precursor to produce free galactose.

Claim 68 (New). The method of claim 62, wherein the host cell during the culturing step secretes an enzyme into the culture medium, which enzyme is capable of degrading the galactose precursor to produce free galactose.

Claim 69 (New). The method of claim 68, wherein the secreted enzyme is a galactosidase.

Claim 70 (New). The method of claim 62, wherein the amplification unit further comprises a nucleotide sequence with a homology to a chromosomal nucleotide sequence of the host cell sufficient to effect chromosomal integration in the host cell of the amplification unit by homologous recombination.

Claim 71 (New). The method of claim 62, wherein the amplification unit further comprises a nucleotide sequence of at least 100 bp with an identity of at least 70% to a chromosomal nucleotide sequence of the host cell.

Claim 72 (New). The method of claim 71, wherein the nucleotide sequence comprised in the amplification unit is a partial non-functional copy of a conditionally essential gene of the host cell.

Claim 73 (New). The method of claim 72, wherein the nucleotide sequence comprised in the amplification unit is a partial non-functional copy of a D-alanine racemase.

Claim 74 (New). The method of claim 62, wherein the polypeptide is a hormone, a pro-hormone, a pre-pro-hormone, a small peptide, a receptor, or a neuropeptide.

Claim 75 (New). A method for constructing a bacterial host cell comprising two or more amplified copies of an amplification unit integrated into the host cell chromosome, wherein the method comprises the steps of:

(a) providing a bacterial host cell wherein a chromosomal gene encoding at least one enzyme involved in the removal of UDP-galactose is non-functional, whereby the host cell is susceptible to inhibition by UDP-galactose endogenously produced by the host cell when the host cell is cultivated in a medium comprising galactose or a galactose precursor;

(b) introducing a nucleic acid construct into the host cell of step (a), the construct comprising an amplification unit, and said amplification unit comprising:

- i) an expression cassette comprising at least one copy of a gene of interest;
- and
- ii) an expressible copy of the chromosomal gene of step (a) or a partial non-functional copy of the chromosomal gene of step (a),

wherein at least one copy of the amplification unit integrates into the host cell chromosome;

(c) cultivating the host cell of step (b) in a medium comprising galactose or a galactose precursor, wherein the at least one chromosomally integrated copy of the amplification unit is duplicated or multiplied on the host cell chromosome; and

(d) selecting a host cell comprising two or more chromosomally integrated amplified copies of the amplification unit.

Claim 76 (New). The method of claim 75, wherein the host cell is a *Bacillus* cell selected from the group consisting of *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*.

Claim 77 (New). The method of claim 75, wherein the chromosomal gene of step (a) encodes an enzyme selected from the group consisting of galactokinase (EC 2.7.1.6), UTP-dependent pyrophosphorylase (EC 2.7.7.10), UDP-glucose-dependent uridylyltransferase (EC 2.7.7.12), and UDP-galactose epimerase (EC 5.1.2.3).

Claim 78 (New). The method of claim 75, wherein the chromosomal gene of step (a) is galE.

Claim 79 (New). The method of claim 75, wherein the galactose precursor is lactose, melibiose, raffinose, stachyose, verbascose or galactinol.

- Claim 80 (New). The method of claim 75, wherein the cultivating medium comprises an enzyme capable of degrading the galactose precursor to produce free galactose.
- Claim 81 (New). The method of claim 75, wherein the host cell secretes an enzyme into the medium which is capable of degrading the galactose precursor to produce free galactose.
- Claim 82 (New). The method of claim 81, wherein the secreted enzyme is a galactosidase.
- Claim 83 (New). The method of claim 75, wherein the nucleic acid construct is a plasmid.
- Claim 84 (New). The method of claim 75, wherein the nucleic acid construct further comprises an antibiotic selection marker flanked by resolvase sites or res-sites.
- Claim 85 (New). The method of claim 84, wherein a host cell comprising a first chromosomally integrated amplification unit is selected after step (b), and the antibiotic marker excised from the host cell chromosome by a resolvase prior to performing step (c).
- Claim 86 (New). The method of claim 75, wherein the amplification unit further comprises a nucleotide sequence with a homology to a chromosomal nucleotide sequence of the host cell sufficient to effect chromosomal integration in the host cell of the amplification unit by homologous recombination.
- Claim 87 (New). The method of claim 75, wherein the amplification unit further comprises a nucleotide sequence of at least 100 bp with an identity of at least 70% to a chromosomal nucleotide sequence of the host cell.
- Claim 88 (New). The method of claim 87, wherein the nucleotide sequence comprised in the amplification unit is a partial non-functional copy of a conditionally essential gene of the host cell.
- Claim 89 (New). The method of claim 88, wherein the conditionally essential gene encodes a D-alanine racemase.

Claim 90 (New). The method of claim 75, further comprising performing one or more additional cycles of steps (c) and (d) using the host cell selected in step (d) in each new cycle; wherein the number of chromosomally integrated amplified copies of the amplification unit increases with each cycle.

Claim 91 (New). A host cell produced by the method of claim 75.